

Sublingual immunotherapy for large local reactions caused by honeybee sting: A double-blind, placebo-controlled trial

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Background: Sublingual immunotherapy (SLIT) proved effective and safe in respiratory allergy, and thus its use in hymenoptera allergy can be hypothesized.

Objective: We sought to assess, in a proof-of-concept study, whether SLIT might potentially be beneficial in hymenoptera allergy. The sting challenge in large local reactions (LLRs) was used to test this hypothesis.

Methods: We performed a randomized, double-blind, placebo-controlled study involving patients with LLRs who were monosensitized to honeybee. After the baseline sting challenge, they were randomized to either SLIT or placebo for 6 months. The treatment (Anallergo, Florence, Italy) involved a 6-week build-up period, followed by maintenance with 525 µg of venom monthly. The sting challenge was repeated after 6 months.

Results: Thirty patients (18 male patients; mean age, 44.5 years) were enrolled, and 26 completed the study, with 1 dropout in the active group and 3 dropouts in the placebo group. In the active group the median of the peak maximal diameter of the LLRs decreased from 20.5 to 8.5 cm ($P = .014$), whereas no change was seen in the placebo group (23.0 vs 20.5 cm, $P =$ not significant). The diameter was reduced more than 50% in 57% of patients. One case of generalized urticaria occurred in a placebo-treated patient at sting challenge. No adverse event caused by SLIT was reported.

Conclusion: Honeybee SLIT significantly reduced the extent of LLRs, and its safety profile was good. Although LLRs are not an indication for immunotherapy, this proof-of-concept study suggests that SLIT in hymenoptera allergy deserves further investigation. Trials involving systemic reactions and dose-ranging studies are needed. (J Allergy Clin Immunol 2008;122:1001-1006.)

Key words: Hymenoptera venom allergy, sublingual immunotherapy, large local reaction, sting challenge

Specific immunotherapy with hymenoptera venom is the cornerstone of the management of hymenoptera venom allergy (HVA) and results in an almost complete protection against adverse (or allergic) reactions from stings in the great majority of patients. The indications, contraindications, and practical rules for venom immunotherapy (VIT) are today well established and accepted by the international medical community. In particular, VIT is indicated in children and adults with a history of previous severe reactions.^{1,2} VIT is given only as subcutaneous injections. Aqueous extracts are preferred, and the duration of the induction phase varies according to investigators and across countries, but there is consensus on the optimal maintenance dose, which is accepted to be 100 µg of venom (and be increased to 200 µg in some patients). The maintenance dose is usually given monthly in the first year and then every 6 weeks or every 2 months in the subsequent years. Although no fatal event has been described with VIT, its administration is considered at particular risk of systemic/severe reactions, particularly with honeybee venom, and it is therefore recommended that the treatment is given with resuscitation/emergency facilities available. The sublingual route for immunotherapy was first proposed in 1986,³ with the aim of improving safety and making the treatment more convenient to the patient. After about 20 years, sublingual immunotherapy (SLIT) has been accepted as a viable alternative to injection immunotherapy for respiratory allergy^{4,5} based on numerous clinical trials⁶ and several meta-analyses.^{7,8} Currently, SLIT is used in clinical practice in many European countries, whereas in the United States there is no product approved by the US Food and Drug Administration.⁹ The major advantage of SLIT resides in its very favorable safety profile, which makes the treatment suitable for home administration. In fact, systemic side effects are rare, severe adverse events are exceptional, and the common local side effects are mild and self-limiting.

Based on these premises, SLIT would in principle be a useful candidate therapy for HVA, but there is no clinical experience at present. This is probably due to the general belief that mucosal immunotherapy is not indicated in a situation in which the allergen is injected. We designed a proof-of-concept clinical trial to explore the effect of SLIT with honeybee venom in patients with HVA. Because of the complete absence of literature and ethical concerns, the experimental model of large local reactions (LLRs), which is a swelling of 10 cm or more lasting more than 24 hours,¹⁰ was chosen for the trial. In fact, it is well known that VIT is able to reduce the extent of LLRs.^{11,12}

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Abbreviations used

HVA: Hymenoptera venom allergy
 LLR: Large local reaction
 SLIT: Sublingual immunotherapy
 VIT: Venom immunotherapy

METHODS**Study design**

The study was designed as a randomized, double-blind, placebo-controlled, 2-parallel group. The enrolled volunteers, all with HVA caused by honeybee venom with LLRs only, were randomized to receive either SLIT or placebo for 6 months. The extent of LLRs was assessed before randomization and after 6 months by means of a standardized sting challenge. Safety and tolerability were evaluated, and specific IgE and IgG antibodies were measured. The study was approved by the reference ethical committee, and all the patients signed an informed consent form.

Patients and diagnosis

Adult (>18 years) subjects of both sexes referred to our units for a suspicion of HVA were enrolled. Inclusion criteria were as follows: (1) ascertained HVA solely caused by honeybee venom and confirmed by using the standard diagnostic procedure; (2) clinical history of an LLR after honeybee stings without any evidence of previous systemic reactions; and (3) willingness to participate in the study. Patients with sensitizations to multiple venoms or other than honeybee venom; who had cardiovascular diseases, systemic immunologic disorders, malignancies, or allergic asthma; or who had lesions of the oral mucosa were excluded. Pregnant women were not admitted either.

The diagnosis of HVA was made according to recommendations and guidelines^{1,2} and involved intradermal tests and the CAP-RAST assay. Intradermal tests were carried out with venom concentrations increasing from 0.001 to 1 µg/mL. All the diagnostic tests were performed with extracts of *Apis mellifera*, *Vespula* species (Stallergenes, Milan, Italy), *Polistes dominulus*, and *Vespa crabro* (Anallergo, Florence, Italy). Serum-specific IgE and IgG levels were assayed with an immunofluorimetric method (CAP-FEIA System, Unicap 100; Phadia, Uppsala, Sweden) for the same allergens listed above. Serum tryptase levels were also measured at enrollment.

SLIT and concomitant treatments

The honeybee venom extract, obtained by squeezing dissected venom sacs (Entomon, Florence, Italy) was the same commercialized and used for injection VIT. It was prepared for SLIT administration (Anallergo) as a 50% glycerinated solution at 3 different concentrations (1, 10, and 100 µg of venom/mL). It was administered in the morning before breakfast, keeping it under the tongue for 2 minutes before swallowing. The induction phase, with gradually increasing amounts of venom, lasted 33 days, as shown in Table I. Afterward, the maintenance dose of 35 µg (7 drops from the 100 µg/mL vial) was taken on alternate days for 6 months. The cumulative amount in the SLIT course was 525 µg per month, which is about 5 times the usual maintenance dose of injection VIT. All patients were provided with autoinjectable epinephrine and prescribed oral antihistamines as rescue medications. A physician was always available for telephone contact in the case of problems with SLIT. The induction phase was performed at the clinic for the first 5 days and when starting with a new vial, whereas the maintenance was self-administered at home. The placebo preparation had the same composition as the active treatment except for the presence of the venom.

Sting challenge

Sting challenges were performed before randomization and after 6 months of treatment. Bees were captured by using Entomon beehives early in the morning and immediately transferred to the hospital, where the sting challenges were performed on the same day according to the method described

TABLE I. The SLIT build-up regimen

Day no.	Vial (µg/mL)	Drops	Dose (µg)
1	1	1	0.05
2	1	1	0.05
3	1	2	0.1
4	1	2	0.1
5	1	3	0.15
6	1	3	0.15
7	1	4	0.2
8	1	4	0.2
9	1	5	0.25
10	1	5	0.25
11	10	1	0.5
12	10	1	0.5
13	10	2	1
14	10	2	1
15	10	3	1.5
16	10	3	1.5
17	10	4	2
18	10	4	2
19	10	5	2.5
20	10	5	2.5
21	100	1	5
22	100	1	5
23	100	2	10
24	100	2	10
25	100	3	15
26	100	3	15
27	100	4	20
28	100	4	20
29	100	5	25
30	100	5	25
31	100	6	30
32	100	6	30
33	100	7	35

TABLE II. Demographic data

	SLIT	Placebo
Sex (M/F)	10/5	12/3
Age (y), mean ± SD	43 ± 15.8	44.6 ± 13.9
Total IgE (kU/L), mean ± SD	189.4 ± 253.1	138.9 ± 150.7
Tryptase (mg/L), mean ± SD	4.9 ± 1.5	7.2 ± 5.4
Honeybee intradermal test (mean of the threshold reactivity in µg/mL ± SD)	0.53 ± 0.43	0.202 ± 0.37
Honeybee-specific IgE (kU/L), mean ± SD	2.2 ± 2.1	3.1 ± 4
Last bee sting before the study (mo), mean ± SD	6.14 ± 3.8	4.4 ± 3.08

M, Male; F, female.

by Golden et al.¹³ The maximal diameter of the LLR was assessed at 30, 60, and 120 minutes and 24 hours after the sting, and the peak diameter was recorded for analysis. The 24-hour measurement was chosen for feasibility and for ethical reasons. All the patients were treated after the 24-hour measurement with antihistamines and steroids and instructed to return if the reaction did not subside. During the challenge procedures, resuscitation facilities were immediately available, and the patients were kept under observation for at least 2 hours.

Statistical analysis

The study was sized to detect at least a 30% change in LLR size with a confidence of 95%, and thus 30 subjects were planned to be enrolled. The

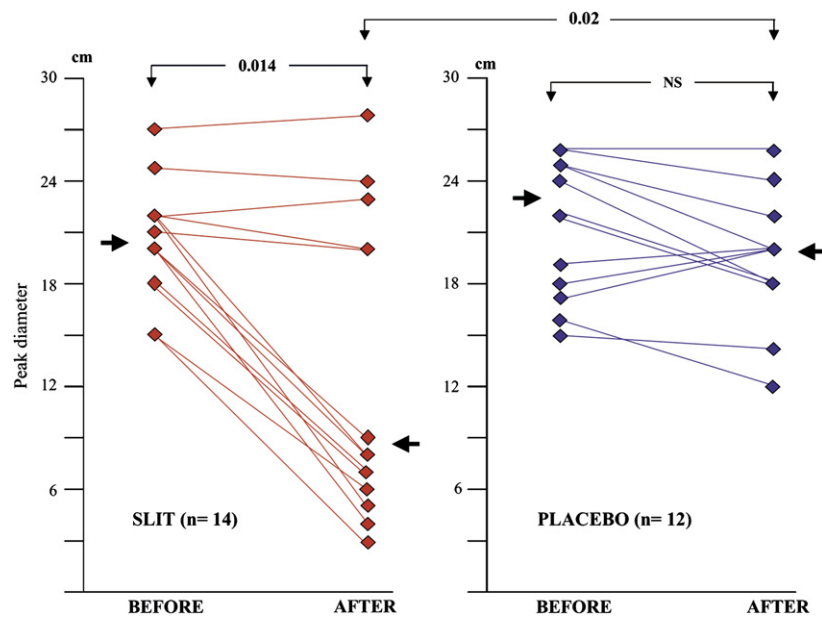


FIG 1. Peak maximal diameter (in centimeters) of the LLR in the SLIT-treated (left) and placebo-treated (right) patients at baseline and after 6 months. Medians are indicated as horizontal thick arrows. Statistically significant differences are indicated at the top. NS, Not significant.

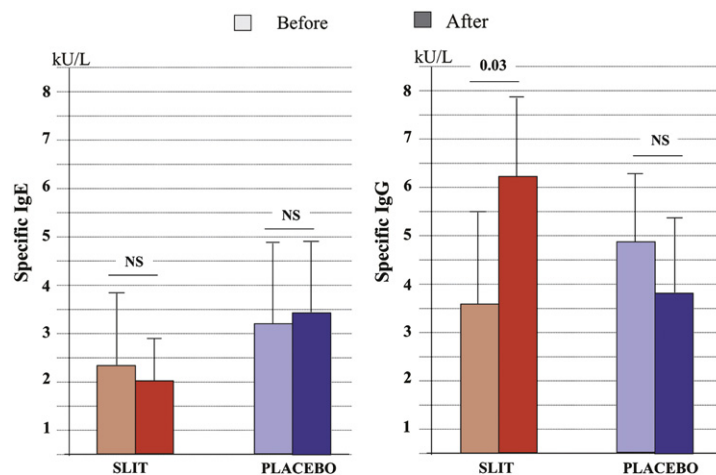


FIG 2. Mean (SEM) honeybee-specific IgE (left) and specific total IgG (right) levels in the active and placebo groups before and after treatment. Values are expressed in kilounits per liter. NS, Not significant.

evaluation parameters in SLIT- and placebo-treated patients, before and after treatment, were compared by means of the *t* test for paired and unpaired samples. *P* values of less than .05 were considered significant.

RESULTS

Thirty adult subjects (18 male subjects; mean age, 44.5 years) fulfilling the inclusion criteria were enrolled and signed the informed consent form. None of them had a history of systemic reactions, and all were monosensitized to honeybee venom. The patients were randomized to either SLIT or placebo, and their demographic characteristics were similar at enrollment, as summarized in Table II. Four patients did not complete the study procedures. In fact, 1 subject from the active group and 3

subjects from the placebo group refused the second sting challenge. The changes in the peak maximal diameter of the LLR in the 2 groups are shown in Fig 1. The peak diameters were comparable at baseline in the 2 groups. A significant reduction could be seen only in the active group after treatment versus baseline ($P = .014$), whereas no change was demonstrated in the control group. Also, after 6 months, there was a significant difference between SLIT-treated and control subjects ($P = .02$) in the peak maximal diameter of the LLR. Of note, 8 of 14 patients had a reduction in their LLR of greater than 50% versus baseline. One of the control patients had a systemic reaction after 15 minutes at the second sting challenge. This reaction was generalized urticaria with initial scalp itching and was successfully managed with intramuscular adrenaline, steroids, and

antihistamines. The serum concentrations of honeybee-specific IgE and IgG before and after treatment are shown in Fig 2. No significant difference was seen between groups and between the 2 time points in specific IgE antibody levels, whereas a significant increase in total specific IgG levels was observed in the active group after SLIT.

During the induction phase, which was performed at the clinic, no side effects were observed by the physicians, and none were reported by the patients. In addition, during the 6-month maintenance phase of immunotherapy, no local or systemic side effects possibly or probably related to the treatment were reported.

DISCUSSION

Today, SLIT is gaining an increasing approval within the medical community as more and more experimental data are published. The good safety profile and the ease of use of SLIT have prompted the investigation of new indications other than allergic rhinitis and asthma. In fact, in the recent literature some studies proposed the possible use of SLIT in conditions such as atopic dermatitis,¹⁴ food allergy,¹⁵ or latex allergy.¹⁶ Despite this, no attempt has been made thus far to use SLIT in HVA, and for this reason, we designed a proof-of-concept clinical trial to assess the potential effects of SLIT. Because of the complete absence of literature on the subject, we had to balance the need for a clear demonstration (or refutation) of the clinical effect and the ethical concerns that usually appear in HVA studies. For this reason, we decided to evaluate the treatment in patients with LLRs, who are at lower risk of systemic or life-threatening reactions,¹⁷ which can be reproduced in a controlled sting challenge. Indeed LLRs *per se* are not an indication for VIT,^{1,10} and this was intended only as an experimental model to approach the problem. On the other hand, the in-field evaluation would have been affected by numerous variables that cannot be easily controlled, whereas the standardized *in vivo* sting challenge allows more reproducible results. Opinions on the utility and safety of the procedure vary,¹⁸ but in the present case a clean model for evaluating the effects was mandatory. Other factors than IgE might influence LLRs, such as a toxic effect of venom proteins,^{19,20} but the patients in this study had the LLR immediately after honeybee stings, and all had positive specific IgE and skin test results with honeybee venom. It is true that LLRs can reach their peak size after 24 to 48 hours, but for practical and ethical reasons, we had to limit the measurement at 24 hours. On the other hand, this measurement was standardized and identical in both groups, so that a bias can be reasonably excluded. Also, no change in the placebo group was seen, and thus the effect of SLIT on LLR is quite clear. Another matter of discussion is the dose of venom used. In clinical protocols the subcutaneous 100- μ g dose is considered effective. On the other hand, the clinical efficacy of SLIT in respiratory allergy has been proved with doses ranging from 3 to 300 times that used in traditional courses of immunotherapy. Because a dose-ranging study was not feasible for practical reasons, a 5-fold dose was arbitrarily chosen.

Our results, under the present experimental conditions, suggest that SLIT might potentially be of benefit also in HVA because the reduction of LLR was clear-cut in the active group. This is in agreement with the results previously described with injection VIT, where the peak diameter was reduced by more than 50% in 56% of patients.¹² As a complementary observation, our trial

confirms that about 4% of the subjects with LLRs are at risk of subsequent systemic reactions, as indicated in literature. SLIT with honeybee venom was well tolerated, and no adverse event or discomfort was reported by patients. Although the safety of SLIT is generally satisfactory, this study was not sized to assess the adverse events. Thus evaluations on larger samples are needed to detect and quantify the occurrence of untoward effects, which might possibly differ in those subjects with systemic venom reactions. Only an increase in honeybee-specific IgG levels was seen in our patients. No change in IgE levels was detected, whereas with subcutaneous VIT, a decrease was previously observed.¹² This might simply be due to the small sample size with large dispersion of the data or to the dose of vaccine used. Moreover, also in respiratory allergy, despite the clinical effect, changes in IgG levels are usually less pronounced than with subcutaneous immunotherapy,^{21,22} and the behavior of IgE is quite variable.

The observed clinical effect of SLIT in HVA is in apparent contrast with the common dogma that injection desensitization is the unique choice for injected allergens, such as hymenoptera venom. Indeed, it has been shown that SLIT can exert a systemic immunologic effect similar to that of injection immunotherapy²³⁻²⁶ and that it can affect also the late-phase response to allergens.^{21,27} Moreover, from a clinical viewpoint, SLIT acts in different target organs, such as the nose, conjunctiva, and bronchi, so that there is no reason to exclude *a priori* an effect in HVA, although there is thus far no study assessing the effects of SLIT in systemic IgE-mediated reactions.

Our results, although encouraging, represent only a starting point derived by using an experimental model. In fact, LLRs are not an indication for VIT, and much has to be done. First, the magnitude of the clinical effect should be confirmed in larger trials; second, an optimization is needed to identify the dose with the best risk/benefit ratio; and third, the efficacy should be assessed in patients with systemic reactions, who are indeed the best candidates to VIT. If all those aspects will be defined with positive results, a larger number of patients would benefit from SLIT for HVA, especially in the case of honeybee allergy, in which immunotherapy has a greater risk of side effects.²⁸

Clinical implications: This trial shows that SLIT reduces LLRs caused by honeybee stings and envisages the possibility of investigating the use of SLIT in HVA.

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